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METHOD OF DIAGNOSTICS OF CHRONIC MICROELEMENTOSES IN FARM ANIMALS

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Introduction

Under the conditions of technogenic transformation of the biosphere, it is of critical topicality to comprehensively examine highly dangerous biogeochemical endemies in animals and humans, their genesis, evolution, prediction of their manifestations as a result of natural disasters and anthropogenic factors. Misbalance of essential and accumulation of toxic chemical elements in animals and humans are known to depend upon their genetic basis and local biogeochemical elemental cycles. The latter are determined by the processes of airing and transformation of matter. Deficiency or excess of specific chemical elements in plants, fodder and in animals causes reduction of their reproducibility and diseases known as microelementoses. The most widely spread of such diseases are cardiovascular and tumor pathologies. Their genesis is mainly attributed to the deficiency of trace elements, for example selenium. Iodine deficiency causes various forms of endemic goiter and cretinism. Excess of strontium, fluorine and molybdenum is responsible for the development of pathologies in bones and joints [4, 8, 17].

At that, **chronic microelementoses** constitute a complex of subclinical and clinical attributes caused by deficiency, excess or misbalance of macro–and microelements in animals attributed to malnutrition of animals and heterogeneity of habitats (table 1).

At diagnosing MTOSes in farm animals and humans and identifying biogeochemical provinces or environmentally unfavorable areas specialists more and more often resort to integrated system research approaches. First of all, this applies to the biogeochemical indication of environmental status of territories. For example, based on critical concentrations of trace elements, their tolerant levels in plants and fodder, there were developed biogeochemical criteria for environmental assessment of territories. Levels and correlations of normed chemical elements in organs and tissues of wild animals are also used at assessing the environmental status of specific trace elements [8]. One of them implies utilization of data on the chemical elemental composition of hair (CECH) that forms depending on the chemical elemental composition of the environment, fodders and general fitness of the body (table 1).

Objectives of the Project :

- Develop an express–method of system diagnostics of chronic microelementoses in farm animals based on the chemical elemental composition of hair;
- Reveal disbalance of standardized macro–and microelements in fodders based on the CECH data;
- Identify natural and man–affected biogeochemical provinces using the data on the elemental composition of hair;
- Develop a technology to produce special fodder additives to correct microelementoses in farm animals.

Table 1. Indicator capacity in relation to trace elements of six selected organs and tissues [2]

Organ, tissue	Zn	Mn	Cu	I	Se	Mo	Cd	Ni	Li	As
Liver	–	+++	+++	+++	+++	+++	++	+	+	+++
Kidney	–	+	–	+++	+	++	+++	++	–	+++
Brain	–	–	+++	–	–	+	–	+	–	+
Rib	+++	–	–	–	–	+	–	+++	+	–
Serum	(+)	–	+	+++	+++	+++	–	(+)	+++	+
Hair	+	+	+	+++	++	+++	+	++	++	++

Composition of hair

On the whole, the chemical composition of hair is determined by genetic, neuro-humoral factors, metabolism and hair formation features, and a number of exogenous factors.

Endogenous factors : metabolism and blood supply features, functioning of sebaceous and sweat glands, neuro-humoral regulation, genetic factors.

Exogenous factors : sticking of mechanical particles (dust) and air aerosols, effects of precipitation and watering, washing using detergents, treating with different actoparasite-killing agents, contamination with feces, urine, soil and bedding particles, living and nutrition conditions.

Technique

The technique comprises the following stages :

1. Collection of data about an animal and its living conditions.
2. Examination of the animal.
3. Taking hair samples.
4. Purification of hair from impurities.
5. Drying the purified hair samples.
6. Grinding of hair samples.
7. Mineralization of hair samples.
8. Instrumental analysis of hair.
9. Data processing.
10. Comparing the data obtained with the range of concentrations of chemical elements ranked according to the environmental status (deficient, normal, excessive).
11. Decision on the status of macro-and microelements and correction of environmentally unfavorable conditions.

First, the following data about an animal need to be obtained : species, breed, sex, age, color, conditions (fatness, lactation stage), type of nutrition and living conditions (pasture, stabling period, etc.). These data are entered in a special sampling card specifying the attributes of a sample (place of sampling, contamination), date of sampling, owner of the animal, address and name of the sample taker.

As a rule, hair samples are taken is a special cattle pen. Samples can be taken by the animal's owner. Places of sampling : withers, middle of back, femur, side under withers, middle of body side, body side under femur, chest, middle of belly, belly under femur, elbow, wrist, knee, heel, bunch of tail. Based on investigations carried out, we recommend using the tail bunch, because its hairs are long, they rarely contain admixtures, it is easy to take samples, when samples need to be large, and concentrations of a number of metals in the tail hairs are higher than in other types of hair.

A sample of hair (about 1.5 to 2 g) is taken along the full length of the tail bunch. A section of the bunch is sheared at a distance of 1-2 cm from the skin by means of scissors (preferably with titanium coating). The sample with a label is placed into a paper or plastic bag and delivered to the laboratory.

Purification of hair is one of the key stages of the technique. Note that there are no unified procedures for hair cleaning from impurities today.

We have performed experiments to prepare and analyze hair samples of cattle in compliance with project objectives. We have looked at the variation of level of chemical elements depending on the degree of hair homogenization (length of fragments cut), temperature of drying, time of washing and other factors [11, 16].

Analytical methods

Different methods are used for quantitative determination of chemical elements in hair: from ICP-mass-spectrometry to electrochemical methods [15]. As applied to the elemental analysis of hair, it is recommended to use mainly atomic absorption (with and without flame). Iodine and fluorine can be detected using ion-selective potentiometry, and selenium can be measured by the specrofluorimetric option and the 'graphite' cell method. In order to improve the validity of data, it is recommended to analyze each time both clean and dirty portions of one hair sample, and a standard sample.

Concentration ranges of chemical elements in hair

The most critical stage of investigations is determining the ranges of concentrations of chemical elements in hair of health animals taking into account their age (calves, mature animals), sex, color and physiological conditions, and animals living in the environment of deficient and excessive macro-and microelements [1, 3, 14].

We have tried to establish such ranges based on literature data and data available at the moment. It becomes clear that there is a definite dependence of CECH on the season and hair color in case of calcium, magnesium, phosphorus and manganese

(Fig.1, table 1). To a smaller degree, this can be observed in the case of copper, zinc and other trace elements (table 2). It is worth noting that there is shortage of data on the levels of selenium, molybdenum, iodine and fluorine in animal hair.

Further tasks

- make a bank of comparative data on CECH of cattle from different regions of Russia with manifestations of microelementoses and from favorable regions;
- correlate CECH with physiological conditions and pathologies;
- correction (prophylaxis) of microelementoses.

Experimental sites and layout

Moscow region, Eastern Transbaikalia and Northern Caucasus were chosen to be experimental sites. It is possible to get samples from Dagestan, Vladimir, Voronezh and Chelyabinsk regions. Environmental and biogeochemical assessment (sampling and analysis of soils, waters, pasture plants, blood of farm animals) of some experimental sites has been performed already.

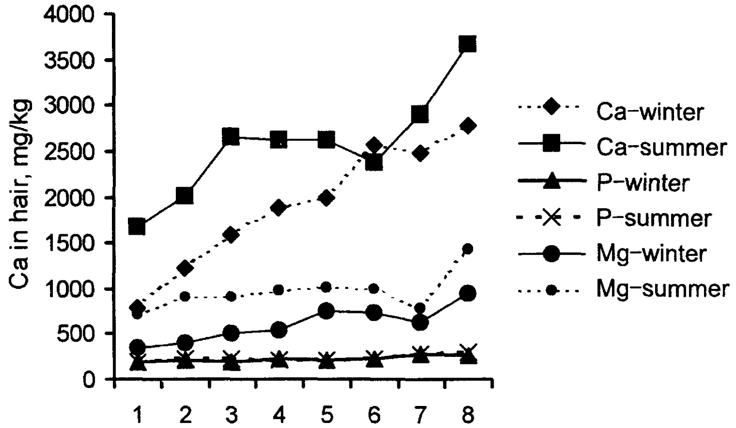

Fig. 1. Change of macro element concentration in cattle hair of graduated colour in winter and in summer by indoor keeping (mg/kg) (cows per colour degree = 50). Graduatrd hair colour : 1– white, 2–7 from yellow to dark red, 8 black [14].

Table 2. Trace element concentrations in cattle hair of graduated colour in winter and in summer by indoor keeping (mg/kg) (Cows per colour degree = 50) [14]

Colour degree+		Mn		Cu		Zn	
		winter	summer	winter	summer	winter	summer
1	mean	4.46^{a}	9.01^{a}	7.83	8.00	119	134
	± SE	1.59	4.69	1.56	2.03	30	48
2	mean	6.73^{b}	8.99^{a}	7.71	8.16	170	223
	± SE	2.97	3.98	1.51	1.59	47	86
3	mean	6.35^{ab}	8.42^{a}	8.04	8.68	172	181
	± SE	3.81	3.77	1.09	1.38	75	78
4	mean	8.30^{c}	10.00^{a}	7.77	8.76	161	155
	± SE	5.30	4.69	1.65	1.40	64	66
5	mean	8.84^{cd}	9.98^{a}	8.61	9.32	140	140
	± SE	5.47	5.22	1.28	1.34	37	42
6	mean	11.45^{d}	10.63^{a}	8.39	8.31	131	139
	± SE	8.49	7.75	1,23	1,36	17	43
7	mean	9.57^{cd}	13.01^{b}	7.43	8.24	123	157
	± SE	6.29	8.99	1.32	1.65	30	76
8	mean	13.11^{d}	19.77^{c}	7.73	9.25	145	138
	± SE	7.34	12.30	1.65	1.76	29	50

+ = graduated hair colour : 1 white, 2–7 from yellow to dark red, 8 black;
abcd = means within each column with different superscripts are significantly different.

Studying the chemical elemental composition of soils, fodder plants and hair of cows during the pasture period on one of the farms in Moscow region has demonstrated that hair of cows can be used for general assessment of the agroecosystem in terms of sustainable development. For example, average levels of copper (5.9 mg/kg), zinc (70 mg/kg) and manganese (5.5 mg/kg) in the analyzed hair samples of the cows on this farm was lower than optimal concentrations. Deficient levels of mobile forms of the above elements have also been detected by means of soil analysis (1.8 mg/kg for zinc; 1.5 mg/kg for copper; 35 mg/kg for manganese).

Table 3. Maximum and minimum concentration of some chemical elements in hair of cattle and man (mg/kg)

Chemical element	Cattle, Russian data		Cattle, data of authors		Cattle, European data		Man, different countries	
	min	max	min	max	min	max	min	max
Ca	480	3148	690	2060	780	3794	50	7100
Mg	232	287	130	990	257	1447	9	252
P	180	590	150	630	174	282	94	273
Fe	33	403	18	156	15	72	4	750
Zn	70	297	84	140	59	223	15	961
Mn	5	59	3	13	1	26	0.2	7
Cu	4	25	7	11	2	14	3	91
Sr	3.4	11.7	0.8	10.8	–	–	0.1	18
Pb	1.0	1.7	–	–	–	–	0.2	13.4
I	0.13	0.20	–	–	0.06	1.65		

In summer 2003, environmental and biogeochemical assessment was performed in the territory of the Tyrnyauz ore field in the vicinity of a molybdenum mining and processing enterprise; samples of soils, plants, waters, blood and hair were taken, specific response of animals at the production cone of ore elements was revealed. In addition, hair samples of cattle were received from other regions of the RF (Dagestan, Chita region, Vladimir region). Data on the chemical elemental composition of soils, waters and plants, which are characterized by increased levels of phosphorus, manganese and strontium and reduced levels of selenium were obtained for some regions in the Eastern Transbaikalia (Chita region).

Correction of microelementoses

The most acceptable ways to correct microelementoses in animals is to use medicines containing trace elements; to control chemical composition of animal diets by means of additives; to produce mixed fodder with controlled content of trace elements; to spray fodder crops with solutions of trace elements; to add compounds of trace elements to the soil of fodder croplands or to block their assimilation by plants. In case of deficiency, most efficient are the methods associated with correction of local biogeochemical cycles of biologically active chemical elements taking into account the genotype of animals and their macro– and microelement requirements by adding deficient essential chemical elements to the soil of fodder croplands. This method is being developed at two experimental sites in Odintsovo district, Moscow region–Moskvoretsk and Nemchinovka farms. Particular attention is paid to the selection of cows and heifers for hair sampling for chemical elemental composition analysis taking into account their age, physiological conditions and the season. Hair samples were taken from the tail bunch, withers and back of animals. The total number of hair samples taken on the Moskvoretsk farm made 160. The number of hair samples taken on the Nemchinovka experimental farm made 38, including 30 hair samples from the tail bunch being in the process of preparation for analysis. Also, hair samples of cattle were taken on private farms near the city of Pokrov and other farms located near Moscow taking into account the status of lactation.

We have tested sodium thiosulfate and recommend using it if it is necessary to neutralize fodder overloaded with mercury, cadmium and lead. Introducing 10g/kg of this substance into the diet of cattle during the period of fattening makes it possible to sharply reduce concentrations of the metals in organs and tissues, and to increase the growth of biomass [9, 10, 12, 13].

Conclusions

On the whole, the problem of CECH as applied to diagnosis of microelementoses is in the focus of keen attention of scientists and active exploration of optimal solutions. Considerable analytical difficulties are associated with the choice of biomaterial, its preparation for analysis and interpretation of data obtained. Using unified hair analysis methods and correlating elemental chemical composition of hair with clinical and subclinical manifestation forms of chronic microelementoses in large areas of the RF will allow enhancing the capabilities of the method as applied to real diagnosis and correction of mineral dysbolism in animals.

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